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CONTROL OF DIASTATIC ACTIVITY IN WHEAT FLOUR

A THESIS SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL OF THE UNIVERSITY OF MINNESOTA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY, 1925

By

REGINALD C. SHERWOOD, M.S.

JUNE, 1925
MINNEAPOLIS

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CONTROL OF DIASTATIC ACTIVITY IN WHEAT FLOUR.

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Introduction

Improvement in the quality of manufactured products, particularly flour, has been the constant aim of the wheat miller. Likewise, the baker has sought to bring about improvement in the foods baked from flour. A comparison of twentieth century milling methods with those of a half century ago leaves no doubt concerning the advances made by the milling industry. Perfection has not been reached, however, and research leading toward the betterment of mill products has by no means been discontinued. In spite of the marked progress in design of mill machinery and development of more refined methods for milling wheat, the miller is still circumscribed in his efforts to produce flour of high quality by the characteristics of the raw material with which he works.

Quality in flour has come to be recognized as a complex characteristic, or rather a combination of several complex factors. Flour strength, the historic definition for which is the ability of a flour to produce large well-piled loaves (Humphries, 1905), can not be ascribed to a single chemical compound or to the physical condition of any chemical entity, but must be considered as a combination of many factors, the proper combinations of these factors determining very largely the quality of the flour.

For many years research has been directed by almost a multitude of investigators toward the determination of those characteristics, whether chemical or physical or both, which are responsible for the strength of wheat flour. In part these efforts have been directed toward the establishment of methods for evaluating flour which will yield results of greater exactitude than the baking test. Many theories have been advanced, some of which have been

A paper based on this thesis has appeared in Cereal Chemistry, Volume 3, page 107-136; 163-182 (1926).

discarded; nevertheless much information has been accumu'ated, with the result that in the light of our present knowledge we recognize certain desirable attributes and can also recognize certain deficiencies. Attempts have been made to overcome some of these deficiencies with varying degrees of success.

Certain sections of the wheat-producing areas have for some time been known to yield wheats which are usually superior in bread-making value. Within these territories there have been noted some localities producing wheat which is ordinarily sound, plump, vitreous in texture, and high in protein, but which when submitted to baking tests does not give entirely satisfactory loaves. This weakness has been attributed by Bailey (1925) to a lack of starch-splitting enzymes which produce available sugars for yeast fermentation. This enzymic action, which is responsible for the hydrolysis of starch to maltose, is defined as "diastatic activity," and the term will be used in this sense throughout this discussion, altho it is recognized that diastases have both liquefying and saccharifying power, the latter, according to Sherman and Schlesinger (1913), to a lesser degree in the case of certain diastases.

In addition to the rôle of diastase in determining the strength of flour, a number of other factors have been recognized as functioning in that connection. These include percentage of protein or gluten, ratio of gliadin to glutenin, "quality" of the gluten as indicated by water-imbibing capacity and in other ways, characteristics of starch, concentration of fermentable sugars, content and character of electrolytes, hydrogen-ion concentration, and buffer value of the flour and dough. The significance of these variables in their relation to flour strength has recently been discussed at length by Rumsey (1922), Sharp and Gortner (1923), and Bailey (1925), and need not be detailed here.

Sugars and Fermentation

The quantity of sugars in sound, normal flour is small. Glucose and maltose, both of which are directly assimilated by yeast, are present normally in flour to a limited extent, usually less than 0.15 per cent (Shutt 1907). Sucrose is present, often approximately one per cent. The total amount of disaccharids and monosaccharids, however, is insufficient food material for the yeast during fermentation of a dough. Consequently sugar must be added when a dough is mixed. While sucrose is used to greater extent than any other sugar, maltose and glucose are also utilized by the

baker. The percentage of sugar used varies widely, from 2 to 5 per cent according to a survey of experimental baking procedures made by Fitz (1924). A report of a large number of commercial dough formulas shows an average of 3.6 per cent sugar in sponge doughs, and 4.0 per cent in straight doughs.

Besides serving as carbohydrate food for the yeast, sugar contributes to flavor, sweetness, and a brown crust, properties which are desired by the American consumer of bread. The amount of sugar ordinarily added in the mixing of a dough is insufficient for these requirements, and it is neither practical nor economical to supply these needs with added sugar. Greater economy is practiced by allowing the diastatic enzymes to hydrolyze the starch of the flour. It is evident that the yeast must depend partly upon the carbohydrate food formed by enzymic hydrolysis, more particularly during the latter part of the fermentation period and the initial portion of the baking period.

The determination of the nature and chemical composition of the enzyme or group of enzymes known as diastase is a many-sided problem which has attracted many researchers. It has long been recognized that the action of diastase upon starch is not a single simple reaction. Starch is first converted into dextrins of varying molecular weights, which are then split up into molecules of maltose. The former reaction has been considered to be the result of hydrolysis by a liquefying enzyme, the latter by a saccharifying enzyme. That these two types of enzymes are present in wheat flour was demonstrated by Baker and Hulton (1908). Because of the two reactions, methods for determining diastatic activity have been founded upon two different principles. The liquefying power is measured with varying technic by the iodine method, which shows the amount of enzyme necessary to convert a given quantity of starch into compounds which do not give a blue color with iodine. The saccharifying power is the enzymic capacity for production of reducing sugar, which is measured by reduction of copper salts to cuprous oxid. In the former determination there is an excess of enzyme reacting upon a definite quantity of the substrate until the starch has been entirely hydrolyzed; in the latter the substrate is in excess, and the amount of the substrate which can be converted to reducing sugar in a given time is measured.

Much of the earlier work upon diastatic activity is conflicting and inconclusive, partly because of the variety of methods which

were employed for extracting the enzymes and determining their power. In the last decade and a half the work of Sherman and his co-workers has contributed substantially to our exact knowledge of amylases. In the first paper Sherman, Kendall, and Clark (1910) reviewed and criticized the methods which have been in use and proposed a new gravimetric method.

According to Kjeldahl's law of proportionality, the reducing sugar formed by action of a known amount of malt extract upon an excess of starch for a definite time is directly proportioned to the amount of actual amylase present, providing that no more than 40 per cent of the original starch is hydrolyzed. Sherman, Kendall, and Clark found that the rate of amyloclastic action was not constant up to the conversion of a considerable percentage of the original starch, but decreased. The importance of activating agents such as sodium chlorid or disodium phosphate was stressed by Kendall and Sherman (1910), since it was found that a purified enzyme preparation acting upon pure starch in pure water gave low activity. Apparently there was no regulation of hydrogen-ion concentration as the activating salts were added.

The amyloclastic and saccharogenic powers of malt and pancreatic amylases were compared by Sherman and Schlesinger (1913) who observed that in the case of pancreatic enzyme the amount of starch digested was about twice the amount of maltose produced, while malt enzyme formed maltose equivalent to two-thirds or more of the amount of starch digested. They suggest as a reason for this difference in starch-splitting and sugar-forming powers that the former expresses the amount of starch completely digested in a given time, while the latter signifies the maltose produced when the enzyme acts on an excess of starch. It is by no means certain that the same relationship between the two activities will hold in the case of a wheat flour dough, since, as mentioned above, the principle involved in making the two determinations is not the same. Concerning the importance of this relationship in wheat flour there is little evidence.

Determination of the nature of the end-products of hydrolysis by diastase (Sherman and Punnett, 1916) proved that glucose as well as maltose was produced. Malt diastase formed only a small amount of glucose. The authors were of the opinion that the glucose originated as a result of amylase action, believing that maltase was not present. The evidence of this conclusion regarding maltase, and the findings of Davis (1916) and Ling and

Nanji (1923) that maltase is present in barley do not harmonize, and it would be presumptuous to state which view is correct. It is worthy of note, however, that Collatz (1922) came to the conclusion that in panary fermentation of wheat flour doughs the amount of glucose formed is insignificant.

That starch as a substrate for enzymic action varies in its characteristics, was demonstrated by Sherman and Baker (1916). Their work showed that starch consists of two components, the more soluble, *B* amylose (or amylose) comprising 20 to 30 per cent, the less soluble, δ amylose (or amylopectin) comprising 70 to 80 per cent. When separated by centrifugal force, δ amylose was precipitated and appeared to consist of swollen grains. Action of three different amylases upon this more insoluble constituent produced less maltose than *B* amylose. It may be that the swollen starch granules of wheat which Whymper (1909) observed to resist digestion during germination were of the δ amylose type.

Sherman, Thomas, and Baldwin (1919) observed that the range of hydrogen-ion activity in which different amylases acted was, for malt pH 2.5 to 9.0, takadiastase pH 2.6 to 8.0, pancreatic pH 4.0 to 10.0, with optima at pH of 4.4 to 4.5, 4.8, and 7.0, respectively.

In the process of purification, Sherman and Neun (1919), and Sherman, Garard, and LaMer (1920) were able to separate amylase from protease only in the case of malt preparations; the best methods of purification of pancreatic amylase did not serve to eliminate proteolytic activity from the product.

It has been reported that amino acids have an influence upon enzyme activity. Sherman and Walker (1919) (1921) and Sherman and Caldwell (1921) (1922a) (1922b) investigated this, using several different amino acids. Glycine, alanine, phenylalanine, tyrosine, and aspartic acid favored enzymic hydrolysis; while histidine and tryptophane did not. Lysine had no effect upon liquefying power, but it did have upon saccharifying power. The favorable results were not due to changes in hydrogen-ion concentration as that was controlled, nor to combination with the products of the reaction. The authors are of the opinion that amino acids exert a protective action upon the enzyme. The deterioration of the enzyme is due in general to its hydrolysis, as it is protein in nature, and the addition of amino acids retards this hydrolysis.

The importance of diastatic activity in relation to baking strength has been recognized for many years. Wood (1907) stated that the size of the loaf depended more upon the diastatic capacity of the flour than upon the sugar content, and particularly upon the capacity for production of gas during the later stages of dough fermentation. Shutt (1907) concurred in this belief. Shortly after these papers appeared, Baker and Hulton (1908) and Ford and Guthrie (1908) reported the results of the first detailed studies of the enzymes of wheat flour. Baker and Hulton asserted that some of the carbon dioxid produced during the fermentation of a dough is formed from the maltose resulting from the action of diastase on the starch. They also demonstrated the presence of proteases in bread dough. The work of Humphries (1910), Swanson and Calvin (1913), Olson (1917), and others confirmed earlier statements that diastatic activity is an important factor in the fermentation of wheat flour doughs. Armstrong (1910) did not believe that diastatic activity was correlated with baking strength, because normal flours, in his estimation, contained an excess of enzymes. He admitted, however, that some flours were deficient in starch-splitting enzymes.

More recently Rumsey (1922), after working out a satisfactory method for determining the diastatic power of flour and studying the effects of several variables upon the determination, studied its relation to flour strength. Rumsey's method is a determination of the saccharogenic power of the diastase, measured by the reducing sugars found in a clarified extract from a flour-water suspension which had undergone autolytic digestion for one hour at 27° C. The diastatic activity is defined as the milligrams of maltose produced by the diastase in 10 grams of flour. Using this method, which will be described in detail later, Rumsey found that temperature exerted a marked influence upon the activity of wheat flour diastase, this activity increasing with the temperature to 63° C., above which there was a rapid decrease. Temperature, he stated, is the most important factor in the control of diastatic activity in the dough. At the usual fermentation temperature the rate of maltose production was found to be nearly constant between two and three hours digestion. The optimum hydrogen-ion concentration for the activity of wheat flour diastase was shown to be pH 4.7, with the optimum range pH 4.0 to 5.3. That this optimum range is barely reached by straight doughs, but is easily reached

by sponge doughs was demonstrated by the work of Bailey and Sherwood (1923).

Rumsey presented a series of curves which showed that the reducing sugar in a dough increased during the first hour of fermentation and then decreased when yeast was present; but when yeast was omitted there was a steady increase in reducing sugar, which was attributed to the hydrolysis of starch by diastases. From his study of fourteen flours of varying source and grade, the author concluded "the flour showing the greater diastatic power should show the greater baking strength and consequently the greater baking value, providing the relative quality and quantity of the gluten is the same."

Sufficient reference has been made to the literature upon diastatic activity to establish its importance as a factor among those which determine the bread-making capacity of flour. As wheat is grown under widely different conditions in the many wheat-producing areas of the world, it is to be expected that diastatic activity as well as protein content, vitreousness, weight per bushel, and other chemical and physical characteristics will vary. Less information is available, however, concerning the diastatic activity of flours from large numbers of wheat samples grown under different conditions. The results which Rumsey (1922) obtained with 9 flours of patent and straight grade from such widely separated sections as Kansas, North Dakota, Ohio, Montana, Utah, California, Alberta, and Saskatoon cover a range in diastatic power from 34 to 304, and are concrete evidence that there are wide variations in this flour characteristic.

In view of this fact it is logical that attempts should be made to supplement the diastatic power in certain cases at least. The agencies for increasing this power are limited. Products made from barley malt, namely malt extract and malt flour, have long been used by bakers, and are known to be of value. Altho there is no direct evidence to support the contention, it is highly probable that these preparations are used indiscriminately, regardless of the natural diastase content of the flour, with the result that in many cases the addition is unnecessary; while in others the amount added is insufficient. Another difficulty is attendant upon the use of malt extract in the bake shop of small output; the diastatic activity of the extract diminishes with age, when the container is opened and the material exposed as in ordinary usage, with the result that the baker must vary the percentage

used in order to contribute the same amount of diastase. This amount can not be estimated readily, as the average baker does not have the facilities for making tests of the extract to determine how much should be used to secure the best results. The enormous quantities of malt extract manufactured each year for use in the bakery have prompted investigation of the effects of the extract upon bread quality.

Collatz (1922) reported an extensive study of the effects upon flour strength of the addition of diastatic ferments and observed that "addition of malt flour and malt extract to doughs increased the volume of the resulting bread. In all cases the use of malt extract gave a superior loaf of bread in volume, grain, and texture, thus increasing the baking strength of the flour."

Two properties of active malt flours and malt extracts tend to reduce their usefulness in bread doughs. These are color and protease activity. Collatz and Racke (1925) have emphasized the hazards attendant upon the indiscriminate use of active or "diastatic" malt extracts in bread making. Their data indicate a progressive darkening of color, and diminution of texture score with increases in the "diastatic activity" of the malt syrup used in the dough batch. It is not yet evident, however, that the disadvantageous effects of active malt extracts can be wholly attributed to the amylases of the extract. We suspect that the active proteases may be responsible for certain of the undesirable effects that have been observed. It is highly desirable, therefore, that a source of amylolytic and saccharogenic enzymes be found which will contribute less to the impairment of crumb color and crumb texture in bread baking.

Wheat, as well as barley, increases in enzyme activity during germination, and is a logical selection as a source for diastatic enzymes. Wheat starch as a substrate for wheat diastase probably has no greater value than other starches, judging from the work of Sherman, Walker, and Caldwell (1919), who found that wheat, corn, rice, and potato starches prepared by the same method did not vary in the rate of reducing sugar production with several different enzyme preparations. Wheat diastase was not used, but the fact that amylases from pancreatin, saliva, barley malt, and *Aspergillus oryzae* gave the same results indicates that wheat amylase would not differ. Germinated wheat or the flour made from it is superior to barley malt flour as a medium for adding

diastase, as the admixture of wheat flour does not reduce the quantity of gluten.

Wheat containing germinated or sprouted kernels is found upon the market not infrequently, in fact, in some seasons the wheat shipped from certain localities very commonly contains sprouted kernels. The federal standards for wheat recognize the hazard in milling wheat of this character by allowing a maximum of 2.0 per cent of damaged kernels in No. 1 grade, 4 per cent in No. 2, 7 per cent in No. 3. While wheat containing sprouted kernels has been milled innumerable times, the milling trade in general considers it inferior in value to sound wheat. Recently there has been a tendency to regard with favor wheat of this nature for blending purposes, and in a few cases which have come to our attention a premium has been paid instead of a discount being demanded for wheat containing small percentages of sprouted kernels. The fact must not be overlooked, however, that wheat which has sprouted while in shocks in the field contains kernels in varying stages of germination, the more advanced stages capable of exerting such a damaging influence that the miller can not be certain of the character of the flour that the wheat will yield.

There have been a number of investigations of the effects of sprouted wheat upon milling and baking quality. These have been reviewed by Bailey (1925).

While most of the reports in the literature dealing with germinated wheat cover experiments made to determine the changes undergone and the effects upon milling and baking, there is record of a patent issued to Dombach (1912) for a process of "treating grain with like grain previously germinated slightly." The process is claimed to produce a high-grade flour from low-grade grain. The grain to be germinated is softened by liquid for 6 to 10 hours, the liquid is removed and the swelling and germination are allowed to take place, with a stream of air forced through the mass by pressure. When the process is completed the germinated grain is mixed with normal grain and the whole mass is brushed, polished, and ground. Special apparatus is described for carrying out these operations. It is mentioned that it is necessary, especially when the grain in question is wheat, that the swelling or germinating process should be rapidly carried out, as wheat easily and rapidly passes over into fermentation.

Summarizing the information contained in the various papers dealing with sprouted wheat, it seems safe to conclude that ger-

minated wheat is a source of enzymes which can be utilized for supplementing the diastatic activity in wheat; that wheat during germination and subsequent scouring in preparation for milling suffers a loss of dry matter, due in part to increased respiration, in part to translocation of food materials to the plumule and rootlets, and in part to dislodging of the embryo; that the addition of sprouted wheat to the mill mix will result in a milling loss in direct proportion to the amount added, but not equal to it; that small percentages of sprouted wheat effect improvement in the flour produced from wheat normally low in diastatic activity, but that large percentages cause impairment of flour quality; that the character of the sprouted wheat, as influenced by the time, temperature, and other conditions under which germination took place has an important bearing upon the quality of the flour. In most of the reported investigations no attention was paid to diastatic activity; in a few, diastase was estimated by the iodine method, which is a measure of the amyloclastic power, and does not give an exact indication of the amount of carbohydrate food available for the yeast during the fermentation of a dough.

Experimental

The problem.—In view of the many facts discussed thus far, the problem of this study was chosen. The object of the investigation was to supplement the diastases naturally occurring in the wheat by the addition, in the milling process, of wheat which had been allowed to germinate for a short time under careful control. By this means an effort was made to regulate the diastatic activity in the flour, and improve, if possible, the baking quality of flour milled from wheat low in diastases by raising the diastatic activity to a higher level. By making the addition during the milling process and varying the amount added according to the diastatic power of the wheat, it should be possible to turn out a finished product satisfactory in its diastase content, thus relieving the baker of the necessity of adding diastatic preparations.

The experimental work may be divided logically into two parts. Before attempting to mill, on a large scale, wheat mixtures prepared as indicated, it was deemed advisable to make a preliminary study of flours milled on a small scale. Accordingly, a program was outlined which included the milling of 2000-gram portions of wheat containdifferent percentages of germinated wheat. Using the results of these trials as a basis for tests with larger quantities, tests with 50-bushel lots were later made on a commercial scale.

Germination of wheat.—The germination of the wheat was carried on in a specially constructed germinator, and as this was used for all the germinating it will be described somewhat in detail. A galvanized tank was secured having an outlet in the hoppered bottom controlled by a valve. A dozen trays were constructed by stretching burlap over a square wooden frame 1¾ inches deep. On the bottom of each tray were nailed cleats which separated the trays, when stacked, by a 2½ inch space. Six trays could be placed in the tank at one time. The wheat to be germinated was spread upon the trays, which were stacked in the tank, and the tank was filled with water. After 4 to 5 hours had elapsed, to allow imbibition of sufficient water for germination, the excess water was drawn off through the outlet. Evaporation from the wheat was retarded by raising the humidity of the atmosphere in the tank. To accomplish this, two inches of water was kept in the bottom of the tank and wet burlap was thrown over the top. The temperature was maintained at approximately 16-18° C. The relationship of temperature to rate of germination was followed by Reynolds (1904), who observed that in wheat incubated at 55-65° F. (13-18° C.) the radicle first appeared in 3 days, while that held at 68-72° F. (20-22° C.) came to the same stage in 2 days.

As the carbon dioxide of respiration, if permitted to accumulate, would affect the rate of germination, it was removed by attaching the outlet pipe of the tank to an aspirator which was operated continuously. Occasional sprinkling and stirring kept the wheat moist and promoted uniform germination.

Red Fife wheat grown at University Farm, St. Paul, was used in the first part of the study. Ten pounds of the cleaned wheat was allowed to germinate as just described, one-half for 3 days, the other half for 5 days. After removal from the germinator, the grain was dried as rapidly as possible in a warm room with a liberal circulation of air provided by electric fans. In less than an hour the sprouts and roots were shriveled, and in 5 or 6 hours the grain was dried sufficiently to store safely. The remainder of the ungerminated wheat and the two portions of germinated wheat were scoured separately in a Eureka scourer, which is designed to remove chaff, dirt, and some of the hairs from the blossom ends of the kernels. In passing through the scourer the germinated wheat lost a portion of the germ, and was separated from the dried sprouts and roots. In cleaning there was naturally somewhat greater loss in the germinated wheat, but the greater part of this loss was material which in the

process of milling would be contributed to the feeds rather than to the flour.

As scouring removed the sprouts and germ, the total protein in the germinated and scoured wheat was somewhat lower than in the normal or ungerminated grain. The difference after 5 days sprouting, was about 0.5 per cent.

Catalase activity of wheat—It has been shown that there is a correlation between catalase activity and some other cell activities, and as this determination can be made easily and rapidly, it was thought that it might serve as an index to the extent of germination. Catalase activity was determined in the ground wheat meal, both ungerminated and germinated, by the volumetric method used by Bailey (1917), modified to conform with the principle advocated by Osterhout (1918) that biological reactions should be measured in terms of the time required to produce a given change rather than the amount of change observed in a given time. Following this principle, the method involved the measurement of the time required to liberate 10 cc. of oxygen from a suspension of wheat meal to which H_2O_2 had been added. The wide-mouthed bottle containing the wheat meal suspension was mounted on a shaker, a miniature wagon attached to an eccentric which was operated by a motor. The agitating device was operated continuously while the reaction was in progress at a speed just rapid enough to keep the wheat meal in suspension. One gram of wheat meal was placed in the bottle, suspended in 100 cc. of water at 20° C. and there was then added 4 cc. of neutralized H_2O_2 capable of liberating 20 cc. of oxygen with excess catalase. The time required to collect 10 cc. of oxygen was measured. The results obtained with Fife wheat are reported in Table I. It is readily apparent from this that there is a regular increase in catalase activity during at least the first five days of germination. This is in agreement with the findings of Choate (1921). It is suggested that this determination may prove a useful index of the extent to which germination has taken place in wheat containing a small percentage of sprouted kernels.

TABLE I

CATALASE ACTIVITY OF RED FIFE WHEAT GERMINATED AND UNGERMINATED

Treatment of wheat	Weight of sample	Time to liberate 10 cc. of oxygen	1/t x 10
	Gram	Min.	
Ungerminated	1	29.00	3.45
Germinated 3 days	1	11.25	8.89
Germinated 5 days	1	7.25	13.80

In order to determine the effect of germinated wheat in varying amounts upon the diastatic activity of flour, ten lots of wheat were

prepared. Two of these consisted entirely of ungerminated wheat to serve as controls; four contained 5, 10, 20, and 40 per cent, respectively, of the wheat germinated 3 days in the manner described; four contained the same percentages of wheat germinated 5 days. Moisture determinations were made upon each lot, and a 2000-gram portion, after tempering to 15 per cent moisture, was milled on a three-stand Allis-Chalmers experimental flour mill. Yields of the feeds and the flour were determined, and the patent flour representing 75 per cent of the total flour was saved for subsequent study.

Milling tests.—The results of the milling tests are shown in Table II. The total flour obtained varies considerably with the different samples, and while the variation is not uniform, since there is a large experimental error in milling tests of this kind, the tendency is toward a lower yield of flour when a large proportion of germinated wheat is added. This confirms the experience of other investigators.

TABLE II

MILLING TESTS OF FIFE WHEAT WITH AND WITHOUT ADMIXTURE OF GERMINATED WHEAT

Lab. No.	Germinated wheat	Total flour	Shorts	Bran	Total feed	Total products
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
			Germinated 3 days			
501	0	74.4	8.9	15.4	24.3	98.7
503	5	72.4	9.2	18.3	27.5	99.9
504	10	73.7	10.1	15.4	25.5	99.2
505	20	73.0	10.2	16.0	26.2	99.2
506	40	73.4	11.4	12.4	23.8	97.2
			Germinated 5 days			
502	0	74.1	7.0	17.2	24.2	98.3
507	5	74.2	8.8	15.6	24.4	98.6
508	10	72.8	6.9	17.7	24.6	97.4
509	20	72.0	8.0	17.7	25.7	97.7
510	40	70.9	7.8	18.6	25.9	96.8

Diastatic activity determinations.—As the prime object of this investigation was the control of diastatic activity, this portion of the work will be discussed first. The method of Rumsey, with slight modifications which did not effect any change in the fundamental principles, was used for all determinations of diastatic activity. This method will be described in detail, for strict adherence to the recommendations of Rumsey determine largely the accuracy of the results. The method involves digestion of a flour-water suspension, clarification of the extract, and determination of its reducing sugar content. A 10-gram sample of flour was weighed out, transferred to a 200-cc. Kohlrausch flask, and brought to 27° C. in a water bath; 100 cc. of water (27° C.) was added, and the flour put in suspension by rotating the flask, after which it was loosely stoppered and placed in the bath

for 60 minutes, with gentle shaking at 15-minute intervals to keep the flour in suspension. At the end of the digestion period, the suspension was diluted to approximately 175 cc. and clarified by adding 3 cc. of 15 per cent sodium tungstate ($Na_2WO_4.2H_2O$) solution, acidifying with 0.8 cc. H_2SO_4 (concentrated diluted 1:1) to a pH of about 2.0, indicated by the pink color produced with 5 or 6 drops of 0.04 per cent aqueous thymol blue (Clark, 1922) solution. This clarification stopped enzymic action immediately and precipitated the protein. After dilution to the 200-cc. mark, the liquid was centrifuged, and a 50-cc. portion of the clear supernatant liquid was removed for the reducing sugar determination.

A blank determination was made upon the same flour to correct for the original reducing sugar content. This was made in a similar manner except that the clarifying reagents were added immediately after the flour was put in suspension, thus halting enzymic action and allowing the estimation of natural reducing sugar in the flour.

Rumsey has discussed thoroly the reasons for the procedure which he adopted. It will suffice to remark here that this method for determining diastatic activity approaches more nearly the actual conditions under which the diastase operates in dough fermentation than any other proposed method. Of the several variables which affect the determination and must be controlled, time, temperature, and concentration of flour are easily regulated by the operator. Hydrogen-ion concentration is determined by the flour, and in normal flours is considerably lower than the optimum for diastatic hydrolysis. Rumsey found that the pH of the different patent flours with which he worked varied from 5.7 to 6.1. Reference to the graph in which he shows the effect of changing the hydrogen-ion concentration, indicates that this variance of 0.4 pH may have had some influence upon the amount of maltose produced, and that adjustment of the pH to the same level in each determination might have changed the relative diastatic power of the series of flours.

Sorenson (1924) commented on Rumsey's work, and criticized his method of estimating diastatic activity for its lack of hydrogen-ion control. Comparing two of Rumsey's flours of the same diastatic power, Sorenson stated that the one with the pH 0.6 higher must have had considerably more power to produce an equivalent quantity of maltose. He suggested determining the buffer value of flour before determining the diastatic activity, thus being in a position to regulate the hydrogen-ion concentration of the flour-water suspension. By using three different concentrations, the method used at the Carlsberg

laboratories, a curve could be constructed and flours compared at a definite pH.

The criticisms of Sorenson are based upon sound reasoning, and a comparison of diastatic power at a uniform hydrogen-ion concentration approaching the optimum is desirable from the standpoint of absolute accuracy. In justification of the method of Rumsey, however, it should be stated that from the practical standpoint the consumer of flour is concerned about what will occur when that flour is made into a dough and baked. Adjustment of the pH to the optimum during the determination of diastatic activity will give a result which may bear no relation to the activity of the enzyme in a bread dough, since bread doughs rarely reach that optimum in their fermentation. The hydrogen-ion concentration and the buffer value of the natural flour influence greatly the result of the diastatic determination, but the same influence is exerted in a bread dough, and a method of estimating enzymic activity which simulates the conditions of dough fermentation will yield the most useful results. Until scientific control of bakery practices advances to the stage that the pH of commercial doughs is regulated accurately, regardless of the pH of the flour used, determinations which will aid in evaluating the flour as it exists will continue to be extremely useful.

Olsen and Fine (1924) have recently contributed an excellent paper which adds considerable to our knowledge of the effects of variables upon diastatic activity. The work of Sherman, Thomas, and Baldwin (1919) showed that the optimum pH for purified malt diastase is 4.5; Collatz (1922) found for the same enzyme in malt extract 4.26; Rumsey (1922) found 4.7 for wheat diastase; the data of Gore (1925) showed an optimum range of pH 4.5 to 5.5, with the highest value at 5.07 for malt diastase. Gore used a polarimetric modification of Lintner's method for estimating the reducing sugar, digested his preparations at 21° C., and controlled the acidity by use of a buffer solution containing acetic acid and sodium acetate. Olsen and Fine stated that the apparent lack of agreement in optimum H-ion concentration is not subject to criticism, but indicated that temperature is an important factor, as different temperatures were used by the different investigators. It was demonstrated that temperature exerts a great influence upon the optimum hydrogen-ion concentration for the activity of malt diastase. They found experimentally the following optima, in terms of pH; 4.3 at 25° C., 5.1 at 45° C., 5.7 at 60° C., 6.05 at 69° C. On constructing a curve from these data they concluded that "the optimum pH appears to be a linear function of the tem-

perature." An equation was derived for calculating the optimum at any temperature:

$$p = \frac{85.5 + t}{25}$$

where p is the optimum pH at temperature t. Several values calculated from this equation coincided with the curve drawn from experimental data. It was suggested that the higher pH at higher temperatures might be explained by greater activity of the hydrogen ions present. They also pointed out that the apparent difference between the optimum for liquefaction and that for saccharification is due to the temperatures at which the determinations were made.

Methods for estimating reducing sugars in a water extract are numerous, and several reliable ones are at the disposal of the researcher. The method of Quisumbing and Thomas (1921) was chosen because in their careful study they have seemingly brought under control all the variables which affect the reduction of Fehling solution. The alkaline tartrate solution contains 65 grams sodium hydroxide and 175 grams Rochelle salts per 500 cc; the copper solution contains 41.2 grams $CuSO_4$, 5 H_2O per 500 cc.

The reducing sugars in the diastatic determinations were measured by the following procedure:

After the clarification of the flour suspension, a 50-cc. aliquot in a 400-cc. beaker, representing 2.5 grams of flour, was neutralized with sodium hydroxide solution and the copper sulfate and alkaline tartrate solutions were added, 25 cc. of each. The beaker, covered with a water glass, was then placed in a water bath maintained at 80° C., for 30 minutes, after which it was removed and the liquid filtered rapidly with suction through a tared Gooch crucible prepared with an asbestos mat. The cuprous oxide was washed with hot water, alcohol, and ether, dried and weighed. The final determination of the cuprous oxide by direct weighing instead of by electrolytic precipitation of metallic copper as recommended by Quisumbing and Thomas has a larger experimental error, but for estimating the result of a biological reaction such as the one with which we are concerned here, it is entirely satisfactory. From the weight of the oxide the weight of the anhydrous maltose was obtained by reference to the tables prepared by Quisumbing and Thomas. The maltose found in the digested flour suspension, less the maltose in the blank, gave the maltose produced by enzymic action. Diastatic activity in Rumsey's units is the milligrams of maltose produced by autolytic digestion of 10 grams of flour at 27° C. for one hour.

The results of the determinations of diastatic activity of the flours milled from mixtures of germinated and ungerminated wheat are given in Table III. Figure 1 shows the same results expressed graphically. Here is conclusive evidence that substantial increases in diastatic power can be produced by incorporating germinated wheat in the mill mix. It will be noted that the addition of 5 per cent of the 3-day germination doubled the enzymic activity, 10 per cent nearly trebled it, but that larger percentages did not result in the same relative increase.

TABLE III

DIASTATIC ACTIVITY OF FLOURS MILLED FROM FIFE WHEAT WITH AND WITHOUT ADMIXTURE OF GERMINATED WHEAT

Flour No.	Germinated wheat	Original maltose per 10 gm. flour	Diastatic activity, maltose produced by 10 gm. flour
	Per ct.	Mgm.	Mgm.
	Germinated 3 days		
Av. 501 and 502	0	22.4	86.4
503	5	41.6	173.2
504	10	37.2	222.4
505	20	45.6	233.6
506	40	120.4	278.8
	Germinated 5 days		
507	5	76.8	237.6
508	10	102.8	266.4
509	20	137.6	327.2
510	40	272.4	385.6

In the case of the 5-day germination, the rate of increase in diastatic activity begins to diminish after 5 per cent is exceeded. Collatz (1922) found the same to be the case with most of the flours to which he added malt extract or malt flour. Rumsey (1922) found that with the flours he studied, those with values in the neighborhood of 250 scored the highest in baking value. A small per cent of germinated wheat was capable of raising the flour in diastatic power to within the range which appears to be desirable. The wheat germinated for 5 days had a much greater influence upon the production of maltose, but further tests of these flours, described later, show that this period of germination was too long.

Reducing sugar originally in the flour is shown in both the tables and the figures. The longer period of germination is responsible for a much larger quantity of sugar in the flour, as well as increased production of sugar.

Changes in protein during germination.—During germination of wheat, many changes take place in the kernel other than those directly related to diastatic activity. Changes in the proteins, especially the gluten proteins, have a particular significance because of the relation-

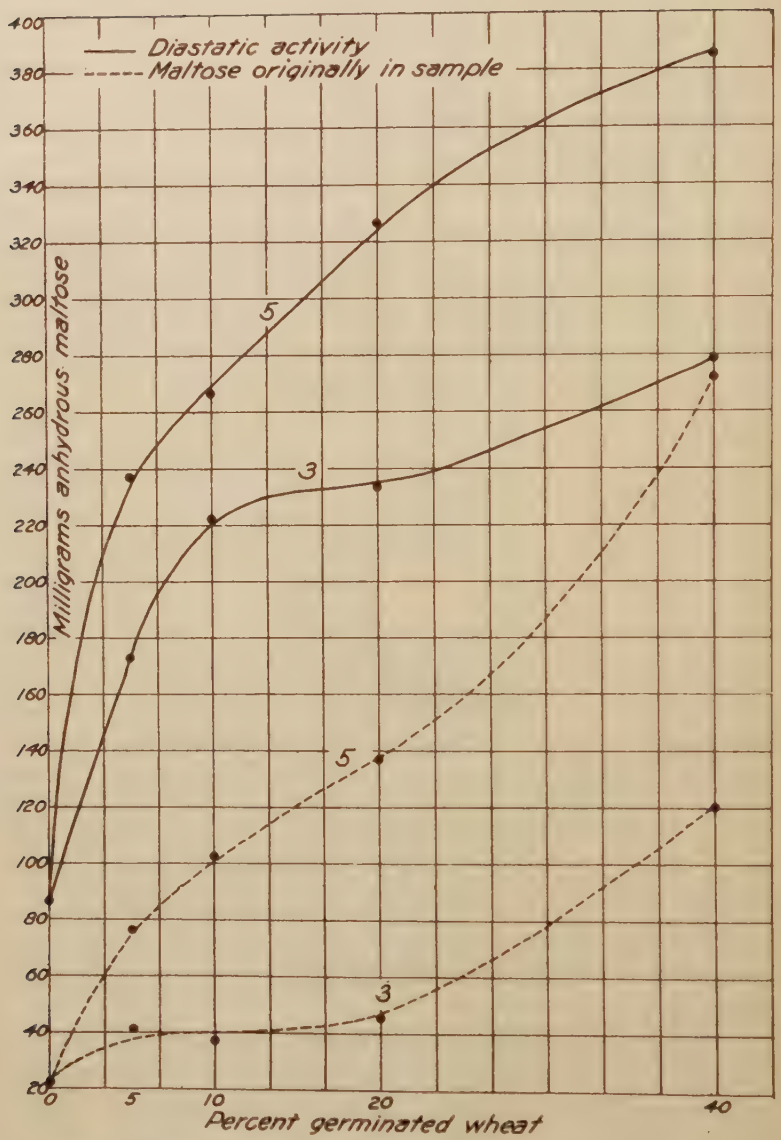

Fig. 1. Diastatic Activity and Original Maltose in Two Series of Flours Varying Percentages of Wheat Germinated 3 Days and 5 Days

ship between both quality and quantity of protein and the baking capacity of flour. The literature contains conclusive evidence that protein-splitting enzymes are more active in wheat which has been allowed to germinate. Balland (1884) found that germinated wheat had the power of liquefying gluten, and considered that the origin of the ferment was adjacent to the embryo. Abderhalden and Schittenhelm (1906) demonstrated that the juice pressed from germinating seeds of wheat was capable of hydrolyzing polypeptides. The work of Vines (1906, 1909), an extensive study of proteases in seeds and other plant tissues, established the presence of proteases in both germinated and ungerminated seeds. Two kinds of enzymes appeared to be active, one peptic in nature, the other ereptic. The ungerminated seeds contained an enzyme which caused hydrolysis of Witte-peptone and acted very slowly upon the reserve protein in the seeds; the germinated seeds elaborated an enzyme capable of digesting fibrin, the activity varying with different seeds. Bialosuknia (1909) also reported that plant proteases active upon plant proteins were present in all seeds germinated from 2 to 5 days.

As the germination of wheat is responsible for increased activity of proteases as well as diastases, some attention must be paid to the effects of the former upon the quality of flour milled from wheat containing a small percentage of germinated kernels. Ford and Guthrie (1908) observed that the addition of a small amount of protease to flour would seriously affect its quality, reducing the tenacity of the gluten and hence the gas-retaining power. They suggested that proteases might be responsible for the unsatisfactory results obtained by the use of some malt preparations. Their findings were in harmony with those of Baker and Hulton (1908), who recognized the deleterious effects of protease action but were unable at that time to demonstrate the presence of proteoclastic enzymes in wheat flour, and attributed the hydrolysis of protein to the enzymes in the yeast. Weaver and Wood (1920) found that traces of pepsin and trypsin added to a dough impaired its bread-making properties.

Large amounts of nitrogen in amino form were considered by Swanson and Tague (1917) to be an indication of certain undesirable qualities in flour. Sorenson's formol titration method of estimating the amount of titratable nitrogen was used as a measure of a certain degree of protein hydrolysis, and it was found that wheat would show increased amounts of titratable nitrogen if exposed to unfavorable conditions so that proteolytic enzymes could attack the protein.

Stockham (1920) demonstrated the presence of proteases in flours of different grades by the liquefaction of gelatin. Flours from sprouted wheats showed higher activity, and less deterioration of protease with age. Both flour and feeds milled from the germ-end half of the kernels liquefied gelatin quite rapidly, while the same products from the blossom end of the kernels liquefied gelatin much more slowly. Sharp and Elmer (1924) concluded from their studies of the proteolytic activity of flours milled from wheats harvested at various stages in their growth that "the proteases of wheat flour are capable of digesting the flour if given sufficient time in which to act."

Protein cleavage studies. —In wheat flour there are always present nitrogenous compounds which are soluble in water. Part of these are protein in nature, part are non-protein and vary in their complexity, including peptids and simple amino compounds. The amounts of these several soluble constituents vary somewhat in different flours. Proteoclastic activity is responsible for hydrolytic changes which will increase the proportion of simpler nitrogenous compounds, and these can be fractionated by the use of certain reagents which are rather specific in their action upon nitrogen complexes. Determination of the nitrogen in the different fractions offers a means of estimating the extent of hydrolysis.

Reagents which can be used for the purpose of fractionating the soluble nitrogen are stannous chlorid solution and copper hydroxid. Scherning (1897) was probably the first to use the former, while the latter was used early by several investigators including Ritthausen (1872), and Stutzer (1881), who described the preparation and use of the freshly precipitated copper hydroxid sludge called Stutzer's reagent. Osborne and Leavenworth (1916) and also Blish (1918) modified the use of this reagent, the latter showing by his work that it precipitated protein and its products of hydrolysis more complex than peptids and amino compounds. Both the tin and the copper reagents were used by Olsen and Bailey (1925), who improved the technic of their use by controlling the hydrogen-ion concentration of the liquid to which they had been added.

To determine whether the addition of germinated wheat would cause an increase in the simpler nitrogeneous compounds in the specially prepared flours, determinations of nitrogen were made following essentially the fractional precipitation method of Olsen and Bailey. A 20-gram sample of flour was suspended in toluene water, diluted to 200 cc., and digested with occasional shaking for 1 hour in a water bath, after which the suspension was centrifuged, and 3 50-cc. portions

of the supernatant liquid were removed. The first was used for the determination of the total soluble nitrogen; to the second in a 100-cc. volumetric flask was added 5 cc. stannous chlorid reagent and sufficient 5 per cent sodium hydroxid solution to bring a faint blue with brom-cresol purple (pH approximately 5.9), after which dilution to 100 cc. was made and 80 cc. was removed for nitrogen determination. To the third in a similar volumetric flask was added 20 cc. 0.2 N sodium hydroxide, phenolphthalein indicator solution, and sufficient 0.2 N. copper sulfate to cause a change from blue to green, followed by dilution to 100 cc. and removal of 80 cc. for determination of nitrogen. Nitrogen was determined by the Gunning modification of the Kjeldahl method. The nitrogen found in the three extracts is reported as total water-soluble, Sn-non-precipitable, and Cu-non-precipitable, respectively.

TABLE IV

TOTAL NITROGEN AND NITROGEN IN VARIOUS FRACTIONS IN FLOURS MILLED FROM WHEAT WITH AND WITHOUT ADMIXTURE OF GERMINATED WHEAT

Flour Lab. No.	Germinated wheat	Total nitrogen	Water-soluble nitrogen	Sn-non-precipitable nitrogen	Cu-non-precipitable nitrogen
	Per cent	Per cent	Per cent	Per cent	Per cent
			Germinated 3 days		
Av. 501 and 502	0	2.091	0.264	0.058	0.020
503	5	2.063	0.267	0.046	0.025
504	10	2.050	0.274	0.069	0.032
505	20	1.993	0.284	0.088	0.032
506	40	2.037	0.315	0.094	0.038
			Germinated 5 days		
507	5	2.017	0.277	0.081	0.029
508	10	1.979	0.285	0.092	0.025
509	20	1.981	0.304	.....	0.031
510	40	1.965	0.404	0.163	0.053

Determinations of total nitrogen in the flours were made at the same time. These results are reported in Table IV. It will be noted that there was a slight decrease in the total nitrogen, and an increase in the fractional nitrogen, more marked in the case of the flours from mixtures of wheat germinated 5 days. Because of the difference in total nitrogen in the flours, the quantities of soluble, Sn-non-precipitable, and Cu-non-precipitable nitrogen have been calculated as per cent of the total, and are expressed as such in Table V. The same results are recorded graphically in Figure 2. The changes in soluble nitrogen are considerable and probably are not due entirely to change in the chemical composition of the protein. The work of Johnson and Bailey (1924) completed since the flours mentioned were studied, established that the quantity of protein nitrogen remaining in the liquid after centrifuging a water suspension of a flour or dough was deter-

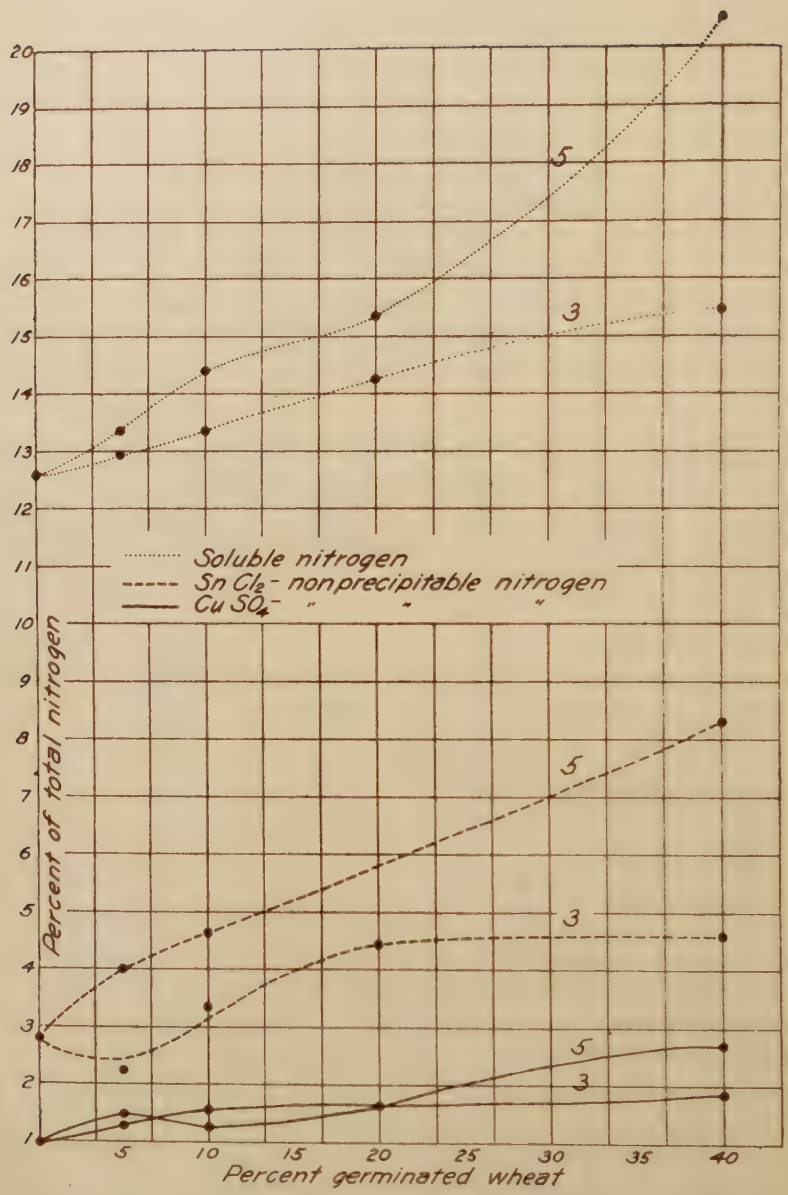

Fig. 2. Effects of Different Amounts of Wheat Germinated 3 and 5 Days upon the Nitrogen in Various Fractions in the Flours Milled from the Wheat Mixtures

mined almost entirely by the hydrogen-ion concentration of the suspension. With the increasing hydrogen-ion concentration (within the limits of the experiment) increasing proportions of the flour protein were dispersed in a form which could not be separated by centrifuging.

TABLE V

NITROGEN IN VARIOUS FRACTIONS, CALCULATED AS PER CENT OF TOTAL NITROGEN IN FLOURS MILLED FROM WHEAT WITH AND WITHOUT ADMIXTURE OF GERMINATED WHEAT

Flour Lab. No.	Germinated wheat	In terms of total nitrogen		
		Water-soluble nitrogen	Sn-non-precipitable nitrogen	Cu-non-precipitable nitrogen
	Per cent	Per cent	Per cent	Per cent
		Germinated 3 days		
Av. 501 and 502	0	12.60	2.80	0.98
503	5	12.94	2.23	1.21
504	10	13.37	3.37	1.56
505	20	14.25	4.42	1.61
506	40	15.46	4.62	1.87
		Germinated 5 days		
507	5	13.73	4.02	1.44
508	10	14.40	4.65	1.26
509	20	15.35	....	1.56
510	40	20.56	8.30	2.70

The flours used in this study were not uniform in hydrogen-ion concentration, and in view of this fact the changes in water-soluble nitrogen have less significance than the changes in nitrogen not precipitated by tin and copper, where the original acidity did not have the same influence. Some difficulty was experienced in obtaining clear extracts with the tin reagent, but none with the copper solution. Comparison of the curves for the 3-day and 5-day germinations shows that considerably more of the products of protein decomposition were contributed by the latter. This would suggest that the gas-retaining powers of the gluten suffered by the admixture of wheat germinated for the longer period. Evidence which follows later supports this contention.

An effort was made to employ changes in viscosity of the acidulated flour suspension as a measure of the extent of proteolysis in the sprouted wheat flour. Such differences in viscosity as were made by the method employed were within the experimental error of the method. A more refined procedure was followed in later studies which will be detailed in another section.

Baking tests.—Flours milled from wheat mixtures containing germinated kernels have exhibited a greater diastatic activity than flours milled from ungerminated wheat. Nitrogen determinations have indicated that undesirable changes in the proteins may have taken place. While such chemical determinations are extremely valuable, and can

be made with a high degree of accuracy, we must still rely upon the baking test of flour to demonstrate in a practical manner how the flour will behave during fermentation with yeast and when subjected to the heat of the bake oven. Baking tests were made with the flours, therefore, using essentially the method described by Bailey (1916). The formula for the dough was as follows:

	Grams	Per cent
Flour	450.00	100.00
Yeast	11.25	2.5
Sugar	11.25	2.5
Salt	6.75	1.5

The doughs were mixed in a mechanical dough mixer for 5 minutes, placed in a fermentation cabinet at 28° C., transferred to a proofing cabinet at 34° C. when two-thirds of the dough representing 300 grams of flour was panned, and baked when ready for the oven.

In order to afford comparisons under the same conditions, the samples of the 3-day series were baked one day and the 5-day series the following day. The results are given in Table VI.

TABLE VI

BAKING TESTS OF FLOURS MILLED FROM FIFE WHEAT WITH AND WITHOUT ADMIXTURE OF GERMINATED WHEAT

Flour Lab. No.	Germinated wheat	Absorption	Fermentation time		Loaf volume	Texture	Color	Expansimeter
			Total	Proofing				
	Per cent	Per cent	Min.	Min.	cc.	Score	Score	cc.
			Germinated 3 days					
501	0	58.8	250	67	1980	99	100	1100
503	5	58.8	245	62	2030	100	100	1050
504	10	58.2	235	60	2080	99	100	1260
505	20	58.2	235	71	2350	101	100	1200
506	40	58.6	230	64	2400	98	100	1020
			Germinated 5 days					
502	0	58.8	260	60	2250	100	100	1010
507	5	58.6	255	55	2350	98	100	1200
508	10	58.0	245	53	2400	95	100	1300
509	20	58.0	255	55	2500	96	100	1250
510	40	58.0	230	47	2350	94	100	1050

Absorption diminished slightly with increase in germinated wheat, altho there was no regular decrease. The fermentation time was shortened appreciably as the amount of germinated wheat was increased to the extent of 20 minutes in the first series and 30 minutes in the second, when the extremes are compared. The majority of this saving in time was in the proofing period, where the higher temperature is conducive to more rapid enzymic action and, therefore, a faster raising of the dough. This is more noticeable in the 5-day series, where the addition of 40 per cent shortened the proofing time nearly one-fourth.

Loaf volumes and expansimeter volumes were increased by the addition of germinated wheat, until the amount added reached 20 per cent of wheat germinated 5 days. The 3-day series showed a fairly regular increase in loaf volume. The 5 per cent mixture gave an excellent loaf of bread, the 10 per cent a larger volume but slightly poorer texture, while the 20 per cent exceeded both of them in texture and volume. The 5-day germination series gave the largest volume, but in all cases the grain and texture suffered. Even the 5 per cent mixture showed somewhat poorer grain and texture than the control, and the others were progressively poorer. None of these loaves was satisfactory and all shrunk upon cooling. Comparison of No. 507 and No. 505 shows that 5 per cent of 5-day germination gave the same loaf volume and diastatic activity as 20 per cent of 3-day germination, but that the former yielded bread with poorer grain and texture, due probably to increased protease activity, resulting in impairment of gas-retaining properties of the gluten. This demonstrated the importance of regulating the length of time which the wheat germinated.

The external color of the loaves was good, a rich brown, somewhat darker with the larger amounts of germinated wheat, indicating that higher diastatic activity, particularly during the proofing period, increased the quantity of sugars which caramelized in the heat of the oven.

It should be mentioned here that it was not anticipated that good baking flours would be produced from wheat containing large percentages of germinated kernels. These larger amounts were used because study of the changes taking place was facilitated when the magnitude of these changes was great.

Determination of hydrogen-ion concentration.—The importance of the hydrogen-ion concentration of flour has been mentioned earlier in this discussion. This determination was made upon the flours milled from germinated wheat mixtures by the conventional electrometric method. The water extract for charging the electrode was prepared by extracting 10 grams of flour with 50 cc. carbon dioxid-free distilled water in a water bath at 25° C. for 1 hour, after which the suspension was centrifuged and the supernatant liquid removed.

Determination of titratable acidity.—Titratable acidity was also determined, by digestion of 18 grams of flour in 200 cc. of carbon dioxid-free distilled water for 1 hour, and titration of a filtered aliquot with 0.01 N. sodium hydroxid using phenolphthalein indicator. The acidity determinations are given in Table VII.

TABLE VII

HYDROGEN-ION CONCENTRATION IN TERMS OF pH, AND TITRATABLE ACIDITY AS LACTIC ACID OF FLOURS MILLED FROM FIFE WHEAT WITH AND WITHOUT ADMIXTURE OF GERMINATED WHEAT

Flour Lab. No.	Germinated wheat	H-ion concentration as pH	Titratable acidity as lactic acid
	Per cent		Per cent
		Germinated 3 days	
501	0	5.99	0.19
503	5	5.98	0.19
504	10	6.01	0.22
505	20	5.92	0.21
506	40	5.89	0.23
		Germinated 5 days	
502	0	6.03	0.21
507	5	5.84	0.20
508	10	5.82	0.20
509	20	5.79	0.21
510	40	5.71	0.23

Inspection of this table shows that there is a significant increase in hydrogen-ion concentration with increasing amounts of germinated wheat. The effect of wheat germinated 5 days is more pronounced. The influence of germinated wheat upon the titratable acidity of the flour is slight, but sufficient to be recognized.

Summary of preliminary experiments.—Summarizing briefly at this point the results of experiments with flours milled from mixtures of normal and germinated wheat, it has been shown that diastatic activity can be increased by the addition of germinated wheat; in fact, can be carried beyond the range which present knowledge indicates is satisfactory from the baking standpoint. The length of the germinating period was found to be important. A small quantity of wheat germinated for 5 days was much less desirable than two or four times that quantity of wheat germinated 3 days. Large quantities of germinated wheat reduced the baking value, probably very largely because of the activity of proteases which were unavoidably contributed with the diastases, or to the presence of a relatively large proportion of partially hydrolyzed gluten. There is evidence that protease activity is not so marked in wheat germinated for the shorter period.

Commercial Scale Milling Experiments

The earlier milling experiments were conducted on a small scale, using 2-kilogram portions of wheat which were ground with the laboratory roller mill. Experiments were then conducted with 3000-pound samples which were milled in the Minnesota State Experimental Flour Mill. This is a 150-barrel flour mill built with small sized commercial machinery, including special devices for determining the losses in cleaning wheat, and the exact yields of all products. Bailey (1922) has described the equipment of the mill and the methods employed for the milling tests of wheat.

Source and description of wheat.—The wheat chosen for the experiments on a commercial scale was of the Turkey Red variety of hard winter wheat grown in Montana. Examination of a sample of the wheat and of the flour milled from a 2000-gram sample on the small experimental mill, showed that it was high in protein and fairly low in diastatic activity, exactly the conditions desired for this study. The wheat graded No. 1 Hard Winter, dockage 1 per cent, protein 13.82 per cent, moisture 10.86 per cent.

Several bushels of the wheat were allowed to germinate for three days, using the apparatus and the method described in the first section. A photograph taken of representative kernels of the germinated wheat appears as Figure 3, and illustrates the length of plumule and rootlets. The wheat was then carefully dried, scoured to remove the sprouts and roots, and preserved for supplementing the diastatic power of the normal wheat.

Milling tests.—Milling tests were made with 3000-pound lots in each instance. Normal, or ungerminated, wheat was milled in order to obtain a control flour, and then a like quantity containing 2 per cent of germinated wheat was milled under the same conditions. It was found most convenient to add the germinated kernels to the normal wheat as the latter was discharged from an automatic, recording scale which dumps 30-pound charges. A predetermined weight of germinated wheat was added gradually

each time the scale dumped, thus insuring that, with the subsequent movement of the grain on its way through the mill to the break rolls, it would be uniformly mixed.

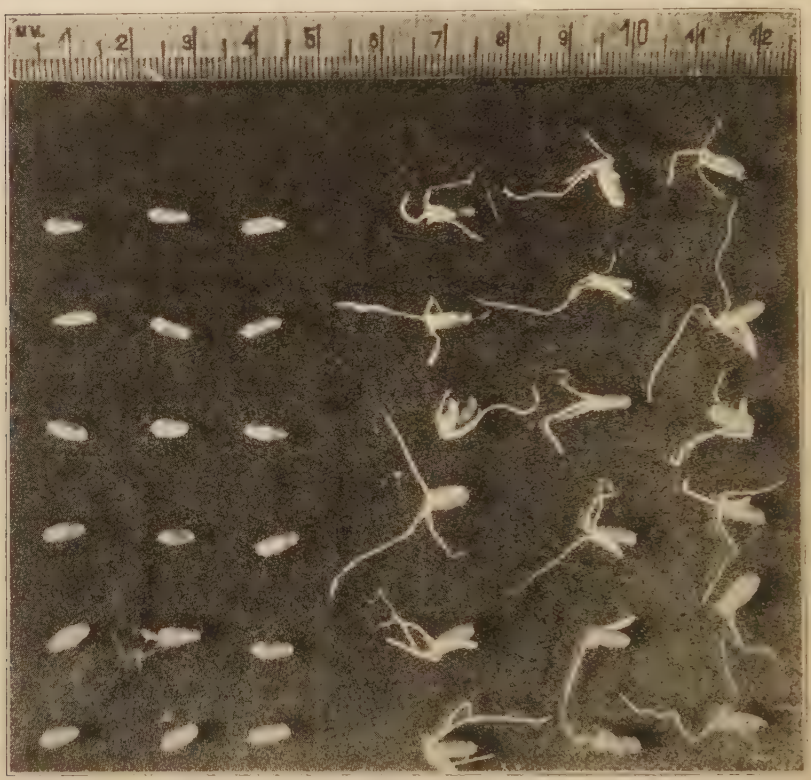

Fig. 3. Hard Winter Wheat Kernels, Ungerminated, and Germinated 3 Days

The wheat was washed, scoured, and tempered, then reweighed on a second automatic scale before milling, in order that the milling yields could be estimated accurately and calculated to the basis of the original moisture content of the wheat. Four products were manufactured, straight grade flour, low grade or red dog flour, bran, and shorts.

Determinations of diastatic activity were made on the flour manufactured in the two milling tests. The results showed that the activity had been increased from 126 in the control flour to

204 by the addition of 2 per cent of germinated wheat, and indicated that 3 per cent could be safely added. Consequently another lot was milled a few days later, in which 3 per cent of germinated wheat was incorporated, and the resulting flour exhibited a diastatic activity of 241 units.

The data collected during the milling tests were recorded and are reported in Table VIII in the form used by Bailey (1922).

TABLE VIII

RESULTS OF MILLING TESTS OF HARD WINTER WHEAT WITH AND WITHOUT ADMIXTURE OF GERMINATED WHEAT

	Per cent	Per cent	Per cent
Germinated wheat	0	2	3
Screenings from receiving separator	0.73	0.73	0.73
Screenings from milling separator	2.30	1.96	2.41
Total screenings corrected	3.02	2.68	3.12
Moisture before tempering	10.8	10.8	10.8
Moisture after tempering	15.0	16.2	15.8
Yield of products calculated to basis of original moisture content of wheat			
Straight flour	76.33	78.02	76.00
Total feeds	27.80	27.48	26.56
Total products	104.13	105.50	102.56

Yields of flour did not vary in order of percentage of germinated kernels. The wheat containing 2 per cent of sprouted kernels yielded more flour than the control, while the 3 per cent yielded nearly the same amount as the control. These data are not indicative of any substantial effect of such small quantities of germinated kernels upon milling yields. Previous tests with larger percentages of germinated wheat showed a reduced yield of flour, and the same results might be anticipated here; but if such is the case, the reduction is too small to be noticeable. No difficulty whatever was experienced in the milling or handling of any of the products. These facts are worthy of mention, since data which follow establish the fact that a decided improvement in the baking qualities of the flour was effected by adding a quantity of germinated wheat too small to be of significance in its effect upon the milling process. The three flours thus manufactured were then subjected to extensive examination in order to determine their relative value.

Determination of diastatic power.—Diastatic activity determinations, made as previously described and recorded in Table IX, showed a regular and substantial increase in the power of producing maltose, with the addition of germinated wheat. It will be noticed that there is a slight increase in the percentage of reducing

sugar in the flour, but that the quantity of reducing sugar produced, reported as diastatic activity in terms of Rumsey's units, was increased more than 60 per cent with the addition of 2 per cent, and nearly 100 per cent with the addition of 3 per cent of germinated wheat. This upper level of diastatic power is within the range which included the best baking flours of Rumsey's series.

TABLE IX

DIASTATIC ACTIVITY OF FLOURS MILLED FROM HARD WINTER WHEAT WITH AND WITHOUT ADMIXTURE OF GERMINATED WHEAT

Germinated wheat	Original maltose per 10 gm. flour	Diastatic activity as maltose produced by 10 gm. flour
Per cent	Mgm	Mgm
0	76.0	126.4
2	79.2	203.6
3	86.0	241.2

Since the protein content of this wheat was entirely adequate for the production of good flour, the acceleration of the mechanism for producing available sugars for yeast fermentation should result in a better quality of flour, as determined by the tests which we apply, providing that there has not been impairment in some other particular. An increase in the gas-producing power of such flour when mixed into a dough may be anticipated but it is important to ascertain also the effects of the addition of sprouted grain upon the gas-retaining power of the gluten proteins. This phase of the problem was attacked from several angles, namely, determination of the gas-producing and gas-retaining power in practical tests with doughs prepared in the usual manner; estimation of the nitrogenous compounds of lower complexity than proteins, as an indication of protein hydrolysis, and estimation of the activity of proteases in digested samples of the flour by titration of amino compounds, using Sorenson's formol titration method; examination of the colloidal properties of the flour proteins, as evidenced by the effects of imbibition upon the viscosity of a flour-water suspension; and finally baking tests of the flours, both in the laboratory and in small commercial quantities.

Gas-producing and gas-retaining power.—Bailey and Johnson (1924b) have devised a method for measuring during fermentation the rate of the expansion of a dough, and also the rate at which carbon dioxid is lost from the dough. Since this method proved to be useful in determining the properties of fermenting dough, it was applied in the study of the flours made from wheat to which varying amounts of germinated wheat had been added.

TABLE X

INCREASE IN VOLUME OF DOUGH, VOLUME OF CARBON DIOXIDE LOST FROM DOUGH, AND SUM OF THESE VOLUMES AS DETERMINED AT 10 MINUTE INTERVALS FOR 250 MINUTES

	Flour No. 894 Control, from ungerminated wheat			Flour No. 895 From mixture containing 2 per cent germinated wheat			Flour No. 912 From mixture containing 3 per cent germinated wheat		
Time	Increase in volume of dough plus CO_2 lost from dough	Increase in volume of dough	CO_2 lost from dough	Increase in volume of dough plus CO_2 lost from dough	Increase in volume of dough	CO_2 lost from dough	Increase in volume of dough plus CO_2 lost from dough	Increase in volume of dough	CO_2 lost from dough
Min.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
0	0	0	0	0	0	0	0	0	0
10	3	3	0	4	4	0	6	5	0
20	9	9	0	10	9	1	13	12	1
30	14	14	1	17	16	1	24	22	2
40	22	21	1	25	23	2	33	31	2
50	31	29	2	33	31	2	44	41	3
60	42	40	2	43	40	3	56	53	3
70	52	49	3	54	51	3	68	65	3
80	62	59	3	64	61	3	81	77	4
90	73	70	3	75	71	4	95	89	5
100	85	81	4	88	83	5	108	103	6
110	96	91	5	100	95	5	122	115	7
120	109	103	6	112	106	6	136	128	8
130	121	115	6	125	118	7	151	141	10
140	134	126	8	139	130	9	166	154	12
150	147	136	11	152	142	10	182	166	16
160	162	148	14	166	153	13	197	175	22
170	175	159	16	179	163	16	212	180	32
180	189	168	21	194	172	22	229	187	42
190	203	174	29	209	178	31	245	189	56
200	217	178	39	223	181	42	260	192	68
210	230	180	50	237	184	53	275	196	79
220	245	183	62	252	185	67	290	201	89
230	259	186	73	266	186	80	307	200	107
240	272	185	87	279	188	91	321	199	122
250	285	188	97	292	192	100	335	201	134

Doughs mixed according to the procedure of Bailey (1916) with 2 per cent of yeast and 3 per cent of sugar, were used for the determinations. The temperature of the water bath was maintained at 28°C. Readings were made every 10 minutes for a period

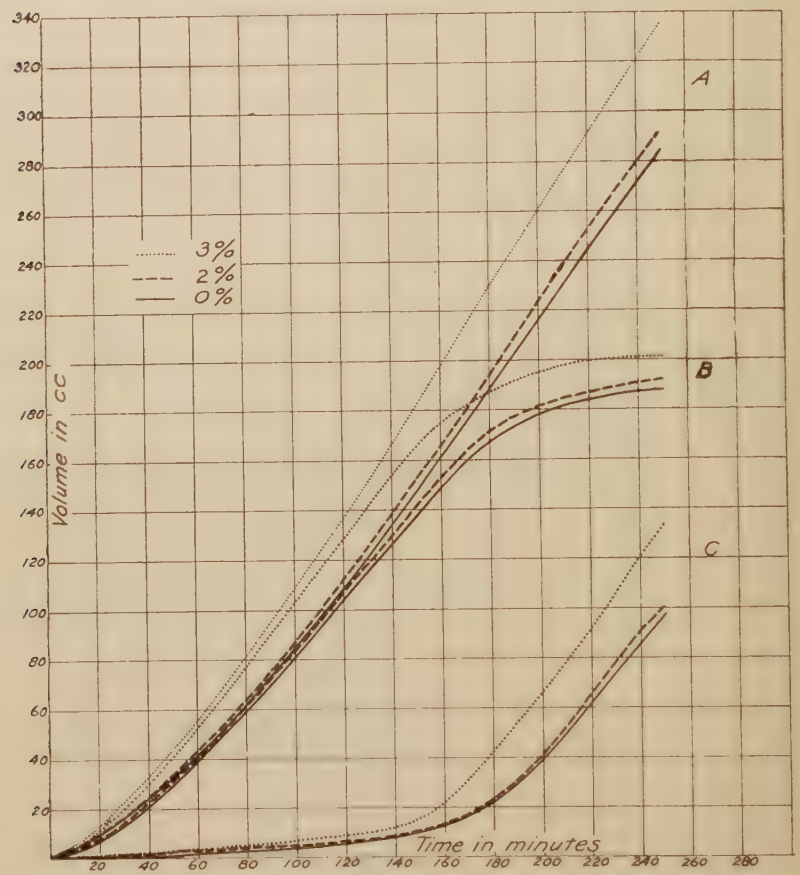

Fig. 4. Changes in volume which occur in systems containing fermenting doughs. Flours Nos. 894, 895 and 912 milled from 0, 2, and 3 per cent of germinated wheat mixtures respectively. Curves A represent the sum of the increase in volume of dough and volume of carbon dioxide lost from the dough; curves B the increase in volume of the dough; and curves C the volume of carbon dioxide lost from the dough

of 250 minutes. The control flour and the flours manufactured from 2 per cent and 3 per cent germinated wheat mixtures were thus compared. The results, which are averages of triplicate determinations, are given in Table X. Curves constructed from this table (Fig. 4) show the results to better advantage. During fermentation the three doughs showed a regular increase in volume

of dough plus gas liberated, the rate of increase being progressively higher with the flours of higher diastatic activity.

The dough from flour containing 3 per cent of germinated wheat began to lose carbon dioxid in quantity about 20 minutes sooner than that from the flour containing 2 per cent of germinated wheat, indicating that the time for the "first punch" would come sooner, and consequently the total time of fermentation would be shorter. The dough prepared with the flour containing 3 per cent of germinated wheat lost a greater quantity of gas in the same length of time, but as more gas was produced, the actual volume of the dough was 13 cc. greater at the expiration of 250 minutes. The dough prepared with the control flour, containing no germinated wheat, began to approach its maximum volume after about 200 minutes, while that with the 3 per cent admixture reached the same volume about 35 minutes sooner.

It is concluded from these gas-production and gas-retention experiments that the addition of 2 and 3 per cent of germinated wheat to the wheat mixture increased the gas producing capacity of the flour and the rate of gas production so that the fermentation time may be shortened and in no way impaired the gas-retaining power, but rather improved it, as a larger volume of dough was obtained.

TABLE XI

TOTAL NITROGEN AND NITROGEN IN FRACTIONS NOT PRECIPITATED BY SN AND CU REAGENTS IN FLOURS MILLED FROM HARD WINTER WHEAT WITH AND WITHOUT ADMIXTURE OF GERMINATED WHEAT

Flour Lab. No.	Germinated wheat	Nitrogen			
		Total	Water-soluble	Sn-non-precipitable	Cu-non-precipitable
	Per cent	Per cent	Per cent	Per cent	Per cent
894	0	2.13	0.236	0.188	0.027
895	2	2.13	0.235	0.193	0.027
912	3	2.10	0.232	0.190	0.031

Determination of nitrogen in fractions not precipitated by various reagents.—In the preliminary trials in which large percentages of germinated wheat were used, it was found that there were appreciable increases in soluble nitrogen, peptid, and amino nitrogen, as determined by fractional precipitation. The larger quantities of simple nitrogen compounds were attributed to protein hydrolysis by proteases added with the germinated wheat. These results indicated, however, that less than 5 per cent of wheat germinated for 3 days would not cause a substantial change in nitrogen distribution. Nitrogen determinations were made, therefore, using the same procedure as outlined previously. The results of the tests are shown in Table XI, where the percentage of each

form of nitrogen in the flour is given. A survey of these data indicated a very slight increase in nitrogen fractions not precipitated by Sn and Cu reagents as the percentage of germinated wheat increased, but the difference was not great enough to be of significance.

Protease activity determinations.—The activity of proteases in the flour was studied, using Sorenson's (1907) formol titration method for determining the extent of hydrolysis during a long digestion of a flour-water suspension. The acidity due to carboxyl groups in compounds existing in the flour was first determined. A 25-gram sample of flour was suspended in 100 cc. of distilled water saturated with toluene, and allowed to stand with occasional shaking for 90 minutes. The suspension was centrifuged, filtered, and 50 cc. of the clear liquid titrated with 0.1 N sodium hydroxide using phenolphthalein as indicator. Then 10 cc of neutralized 40 per cent formaldehyde was added, and a second titration made with 0.1 N sodium hydroxide. The difference between the two titrations indicated the quantity of alkali required to neutralize the free carboxyl groups. Two samples of each flour were then suspended in water in the same manner and digested, the first for 46 hours at 35°C. and the second for 6 days at 35°C. Determinations of acidity were made by the method just described.[2] These results are recorded in Table XII.

TABLE XII

PROTEASE ACTIVITY OF HARD WINTER WHEAT FLOURS MILLED FROM WHEAT WITH AND WITHOUT ADMIXTURE OF GERMINATED WHEAT, AS DETERMINED BY FORMOL TITRATION AFTER AUTOLYTIC DIGESTION OF 1:4 FLOUR-WATER SUSPENSIONS

Flour Lab. No.	Germinated wheat	0.1 N sodium hydroxide required to neutralize extract of 25 grams flour		
		Undigested	Digested 46 hours	Digested 6 days
	Per cent	cc.	cc.	cc.
894	0	1.3	2.0	3.6
895	2	1.3	2.1	3.8
912	3	1.3	2.2	3.9

Inspection of these data shows that there is a definite increase in the amount of sodium hydroxide required to neutralize the extract after 46 hours digestion and, as would be expected, a greater increase after 6 days digestion. This shows that proteases are present in the flours. Comparing the three flours, however, it is seen that flours 895 and 912 required no substantial increase of alkali for neutralization in either of the series of digested samples.

The results indicate only a slight increase in protease activity in the flours milled from wheat mixtures containing 3 per cent or less of germinated wheat after a long digestion. It should be

2 Acknowledgment is made of the kind assistance of Mr. Andrew Cairns.

mentioned that in bakery practice the doughs never stand this length of time; 5 hours or less is the usual fermentation time of bread doughs and, during this comparatively short interval, only small changes in the amount of titrable nitrogen would be anticipated.

Activity of enzymes is influenced by the ratio of flour to water as well as by the time of digestion. It is certain that the 1:4 suspension used for the tests of protease action will permit more rapid hydrolysis than the 1:0.6 dough prepared for bread purposes. The conclusion is drawn from this study of protease activity that the amount of proteases contributed by the germinated wheat used in preparing these flours is insignificant in its effect upon bread doughs.

Viscosity determinations.—A further indication of the effects of the addition of germinated wheat upon protein quality may possibly be indicated by viscosity determinations, using the method of Gortner (1924). This method involves washing a definite weight of flour, first with 1000 cc. of distilled water with agitation at 15-minute intervals for 1 hour, decantation of the supernatant liquid when the insoluble residue has settled, and then a second washing with 500 cc. of distilled water, after which the residue from decantation is diluted to exactly 100 cc. A measured portion of this thoroly mixed suspension is transferred to the cup of the viscometer, and the reading taken.

Concentrations of 12, 15, 18, and 21 grams were examined in this manner, and from the logarithms of the results the constants *b* were determined. Table XIII contains both the actual reading in degrees MacMichael and the constants *b*.

TABLE XIII

VISCOSITY AS DEGREES MACMICHAEL AND CONSTANT *b* OF FLOURS MILLED FROM HARD WINTER WHEAT WITH ADMIXTURE OF GERMINATED WHEAT

Observations Made Upon Washed Flour Suspensions of Varying Concentrations

Flour Lab. No.	Germinated wheat	Viscosity				Viscosity constant *b*
		Flour Concentration gm.				
		12	15	18	21	
	Per cent	M°	M°	M°	M°	
894	0	27	50	84	124	2.73
895	2	28	55	90	142	2.88
912	3	22	42	67	115	2.87

The flours milled from the wheat containing germinated wheat gave slightly higher results, expressed as constant *b*, but the differences are so slight as to indicate no appreciable change in such gluten properties as are measured by this method of examination.

Baking tests in the laboratory.—The flours were subjected to baking tests in the laboratory, following the method described by Bailey (1922) and using the following formula:

	Grams	Per cent
Flour	350	100
Yeast	10.5	3
Sugar	10.5	3
Salt	5.25	1.5
Fat	5.25	1.5
Water	sufficient	

In Table XIV the results of the tests are listed.

TABLE XIV

LABORATORY BAKING TESTS OF FLOURS MILLED FROM HARD WINTER WHEAT WITH AND WITHOUT ADMIXTURE OF GERMINATED WHEAT

Flour Lab. No.	Germinated wheat	Fermentation period	Loaf volume	Color	Texture
	Per cent	Min.	cc.	Score	Score
894	0	340	2000	97	98
895	2	332	2100	98	99
912	3	323	2150	99	100

The fermentation time was shortened somewhat with the germinated wheat samples, and it was in the proofing period that most of the time was saved, as the diastatic enzymes were accelerated at the higher temperature of the proofing thermostat with more rapid production of carbon dioxid. The loaf volume was progressively larger with an increase in the proportion of germinated wheat from 2 to 3 per cent. An improvement was noted in color and texture of the crumb, the difference in color being attributed to the larger volume and improved texture, which result in greater dispersion of colloidal particles and consequent lighter color. The better texture is evidence that the quality of the gluten was at least as good, if not slightly better in the germinated wheat flour. Somewhat browner crusts were obtained with Nos. 895 and 912 because of the larger amount of sugars present when the loaves were ready for the oven. Photographs of cross-sections of the loaves are shown in Figure 5.

Reducing sugar in fermenting doughs.—In order to determine whether the addition of germinated wheat was responsible for production of reducing sugars in greater quantity during fermentation of a dough, flours 894, and 912 were doughed in the usual manner with 3 per cent yeast and 3 per cent sugar. The total reducing sugar calculated as maltose was determined immediately after mixing, at the end of two hours, and after five hours. These results are given in Table XV.

TABLE XV

REDUCING SUGAR AS MALTOSE IN FERMENTING DOUGHS

Flour Lab. No.	Germinated wheat	Maltose at intervals during fermentation		
		Milligrams per 10 grams dough		
	Per cent	When mixed	2 hours	5 hours
894	0	338	430	292
912	3	402	484	380

The 3 per cent flour contained 64 milligrams more maltose than the control at the outset, and 88 milligrams more at the end of 5 hours. This justified the conclusion that there was more fermentable sugar available for the yeast in the dough made from germinated wheat flour throughout the fermentation period. Likewise, at the end of the fermentation period there was a larger quantity present, which indirectly contributed to greater expansion in the oven and browner crust. Reference to Figure 4 will show that No. 912 produced considerably more carbon dioxid than No. 894, evidence that more sugar had been utilized by the yeast. This greater consumption of sugar, coupled with the fact that a larger quantity of sugar still remained in the dough at the end of fermentation, is substantial proof that the mechanism for producing available sugar has been improved by the addition of germinated wheat.

No. 894 No. 895 No. 912

Fig. 5. Bread Baked in the Laboratory

Baking tests on a commercial scale.—Recognizing that conclusions based upon baking data secured only in the experimental laboratory where one-loaf doughs are mixed individually might be open to criticism, it was deemed advisable to bake these flours on a commercial scale. This work was done in the baking department of Dunwoody Institute. The operations were in charge of an experienced baker who also judged the finished loaves. No dif-

ficulty was encountered in the mixing or handling of any of the doughs. The same fermentation time was allowed in each case, but the proofing time was shortened with the germinated wheat flours. Nos. 894, 895, and 912 were proofed 85, 80, and 67 minutes respectively. The flours from germinated wheat mixtures showed better oven spring, No. 912 excelling No. 895 in this particular. Loaf volume, texture, and more particularly grain were favorably influenced by the addition of germinated wheat. The bread from the flour milled from the 3 per cent mixture, No. 912, was classed by the scorer as exceptionally good, superior to that from the control flour. The values assigned by the scorer are given in Table XVI.

TABLE XVI

RESULTS OF BAKING TESTS AT DUNWOODY INSTITUTE OF FLOURS MILLED FROM HARD WINTER WHEAT WITH AND WITHOUT ADMIXTURE OF GERMINATED WHEAT

	Perfect score	Flour No.		
		894	895	912
Germinated wheat, per cent		0	2	3
1. Loaf volume	10	9½	9½	9¾
2. Color of crust	8	7¼	7¼	7
3. Symmetry of form	3	2¾	2½	1¾
4. Eveness of bake	3	2¼	2½	2½
5. Character of crust	3	2½	2¾	2½
6. Break and shred	3	1	2	2¾
7. Color of crumb	10	10	10	10
8. Texture	15	13	14½	14
9. Grain	10	8½	9¾	10
10. Taste	20	18½	15	16½
11. Flavor	15	14	14	14
Total bread score	100	89¼	89¾	90¾

Hydrogen-ion concentration and titratable acidity.—The influence of hydrogen-ion concentration upon the determination of diastatic activity and upon the quality of flour in general has been mentioned. Because of the importance of this characteristic, determinations were made by the procedure used for the preliminary study. At the same time the titratable acidity was determined. The results are reported in Table XVII.

The degree of acidity expressed both as pH and titratable acidity calculated as lactic acid was found to be practically the same in flours with and without germinated wheat. It is safe to conclude, therefore, that none of the comparisons made in the study of the flours was influenced by a difference in acidity.

Effects of aging the flours.—Aging of sound, high grade bread flours is known to improve baking quality, particularly during the first four or five weeks. The results of numerous investigations

have recently been reviewed by Bailey (1925). The general conclusion seems to be that loaf volume, color, and absorption improve during the first few months, but that loaf volume may diminish after extended storage, even tho the color and absorption continue to improve. Proteolytic activity gradually decreased in storage, sprouted wheat flours retaining their activity in storage for a few years. Mangels (1924) found that there was in general an increase in diastatic activity during storage. Both common spring and durum wheat flours gave smaller loaf volumes after several months storage.

TABLE XVII

HYDROGEN-ION CONCENTRATION AS pH AND TITRATABLE ACIDITY AS LACTIC ACID IN THE FLOURS MILLED FROM HARD WINTER WHEAT WITH AND WITHOUT ADMIXTURE OF GERMINATED WHEAT

Flour Lab. No.	Germinated wheat	pH	Titratable acidity as lactic acid
	Per cent		Per cent
894	0	6.12	0.13
895	2	6.13	0.13
912	3	6.14	0.14

Bailey and Johnson (1924a) reported observations of hydrogen-ion concentration during aging which show that the increase is quite rapid for the first few weeks, and then more gradual for many months. In a study of changes in hydrogen-ion concentration of wheat and its products stored under different conditions of temperature and humidity, Sharp (1924) found that the acidity increased, and, in general, more rapidly at higher temperatures and higher moisture content.

The flours used in this study were examined after 12 months storage. At the time the flours were milled, portions of each were placed in tin cans and stored in a refrigerating plant maintained at 7° to 10°C. Other portions were stored in sacks in the State Experimental Flour Mill warehouse, the temperature of which fluctuated with the temperature of the outside atmosphere since it is not artificially heated.

Effects of aging upon hydrogen-ion concentration and titratable acidity.—Hydrogen-ion concentration and titratable acidity were determined in the same manner as before, the results appearing in Table XVIII.

The hydrogen-ion concentration increased with aging, the amount of increase depending upon the conditions of storage. In

cold storage the change was about 0.08 pH and in mill storage about 0.16 pH. The germinated wheat did not seem to influence the rate of change, as the three flours showed practically the same increase. The greater increase in the samples stored in the mill is probably due to the fact that the high temperature during the summer, often 35°C. (95°F.), induced more rapid changes.

TABLE XVIII

EFFECT OF AGING UPON THE HYDROGEN-ION CONCENTRATION AND TITRATABLE ACIDITY OF FLOURS MILLED FROM HARD WINTER WHEAT WITH AND WITHOUT ADMIXTURE OF GERMINATED WHEAT

Flour Lab. No.	Germinated wheat	Hydrogen-ion concentration			Acidity as lactic acid		
		Aged 1 month	Aged 12 months		Aged 1 month	Aged 12 months	
			Mill warehouse storage	Cold storage (7°-10°C.)		Mill warehouse storage	Cold storage (7°-10°C.)
	Per cent	pH	pH	pH	Per cent	Per cent	Per cent
894	0	6.12	5.95	6.05	0.13	0.14	0.14
895	2	6.13	5.98	6.04	0.13	0.14	0.13
912	3	6.14	5.98	6.06	0.14	0.14	0.14

The titratable acidity calculated as per cent lactic acid showed with one exception a very slight increase, not enough to be significant. The conditions of storage did not appreciably affect the changes.

Effect of aging upon diastatic activity.—Diastatic activity of the flours determined after 12 months storage is shown in Table XIX. Storage conditions were the same for the samples tested for acidity. (Table XVIII.)

TABLE XIX

EFFECTS OF AGING UPON THE DIASTATIC ACTIVITY OF THE FLOURS MILLED FROM HARD WINTER WHEAT WITH AND WITHOUT ADMIXTURE OF GERMINATED WHEAT

Flour Lab. No.	Germinated wheat	Diastatic activity			
		Original	Aged 12 months		Aged 26 months
			Mill warehouse storage	Cold storage (7°-10°C.)	Mill warehouse storage
	Per cent				
894	0	126	136	130	100
895	2	204	210	208	167
912	3	241	242	243	178

The results indicate no pronounced change in diastatic activity after one year storage, such differences as were noted being insignificant. A portion of each sample was stored in the warehouse an additional 14 months, or a total of 26 months. Diastatic activity of these samples was appreciably lower than in the fresh flour. They remained in essentially the same relation to each other however, the flours milled from germinated wheat mixtures being higher in diastatic activity than the control flour.

Baking tests and washed gluten tests of aged flours.—In order to obtain the opinion of an experienced baker who did not know the nature of the flours, and to learn whether germinated wheat would cause deterioration in the flour on aging, two of the flours, Nos. 894 and 912, milled from the ungerminated wheat and the 3 per cent germinated wheat mixture respectively, from the mill storage, were sent to a large research laboratory without a description of their method of manufacture. Baking tests were made when the flours were 12 months old, using a formula which called for 1 per cent of yeast. No. 912 gave 3.25 per cent greater loaf volume than No. 894 and slight improvement in color. There was no evidence that the presence of the germinated wheat flour in the mixture (No. 912) had resulted in any deterioration after storage for a year. Washed gluten tests were made at the same time. No. 894 had a slightly grayish gluten, not so bright as No. 912, which was slightly yellow and also a trifle more resistant. Both samples showed a very good quality of gluten.

Five months later, or 17 months after they were milled, the same samples were again baked in the same laboratory. The absorption was increased by one per cent in both flours during the 5 months. No. 912 did not mature so fast, but exceeded No. 894 in loaf volume, this time 6.25 per cent, and showed slightly more even and velvety texture. Both flours were considered good, with preference expressed for No. 912, and no deterioration in the latter was evident from these tests.

Baking tests were also made at the State Experimental Flour Mill after the flours had been aged for 17 months. The same procedure and formula were followed as in the earlier baking trials in the same laboratory. The results given in Table XX are interesting in several respects. The different conditions of storage did not produce the same effects. The mill storage brought improvement while the cold storage was evidently responsible for deterioration, especially in point of texture. In the cold storage samples, the color was nearly the same in all three flours, but was of a creamy tint; less bleaching of the carotinoid pigments had taken place. The loaf volume of No. 895 was 2½ per cent smaller, the others 1 to 3 per cent larger, than when the flours were fresh. The same order of volume was maintained as in the fresh flours, however, namely, larger volumes with increasing proportions of germinated wheat. No tendency toward a greater deterioration of flour milled from the germinated wheat than from the control or ungerminated wheat could be detected under either condition of storage.

TABLE XX

EFFECTS OF AGING FOR 17 MONTHS UNDER DIFFERENT CONDITIONS OF STORAGE UPON THE BAKING QUALITY OF THE FLOURS MILLED FROM HARD WINTER WHEAT WITH AND WITHOUT ADMIXTURE OF GERMINATED WHEAT

	Mill storage			Cold storage		
Flour No.	894	895	912	894	895	912
Germinated wheat, per cent	0	2	3	0	2	3
Absorption, per cent	62.3	62.3	60.0	62.0	60.6	60.6
Loaf volume cc.	2200	2400	2450	1950	2120	2210
Texture, score	100	100	100	94	95	92
Color, score	99	101	101	99	99	99

The samples stored in the mill warehouse showed a marked improvement in baking quality after 17 months aging. From the standpoint of loaf volume, improvement was greater in the flours milled from germinated wheat mixtures. No. 894 increased 200 cc with aging while Nos. 895 and 912 each increased 300 cc. The color was better in the two containing germinated wheat flour, the texture was the same. Absorption increased more with aging in the first two flours.

The baking tests warrant the conclusion that the flours milled from wheat containing 2 or 3 per cent of kernels germinated for a short time under careful control improved in baking quality with age, and after storage for nearly a year and a half showed no signs of deterioration. The flours made from the germinated wheat mixtures evidenced greater improvement than the flour from normal wheat. All three made excellent bread.

Summary and Discussion

Altho the results of each portion of this investigation have been discussed briefly as the data were reported, it appears advisable to recapitulate and summarize the results of the study as a whole.

In the first part of the work wheat which had been allowed to germinate for 3 days, and another portion which had germinated for 5 days were mixed with normal wheat in varying amounts and the several samples were milled on an experimental flour mill. Flour was produced which showed marked increase in diastatic activity, depending upon the quantity of germinated wheat in the mixture. The relative diastatic activity was increased from 86 in the control to 385 in the flour milled from a mixture containing 60 per cent of ungerminated wheat and 40 per cent of wheat germinated for 5 days. This large activity is probably entirely

unnecessary, and further tests showed that the inclusion of sufficient sprouted wheat to effect it was undesirable. As little as 5 per cent of germinated wheat was sufficient to double the diastatic activity. Larger percentages did not increase it in the same ratio.

The length of time which the wheat germinated was found to be a most important factor. The addition of 20 per cent of wheat germinated for 3 days resulted in the same diastatic activity as 5 per cent of the wheat germinated for 5 days. While the former made a good loaf of bread, with large volume and good texture, the latter when baked gave large volume with poor texture, showing that the gas-retaining capacity of the flour had been reduced.

Flours milled from wheat mixtures containing 10 per cent or more of the wheat germinated 5 days gave unsatisfactory results when baked, and the flour from the 40 per cent mixture had lost so much gas-retaining power that the loaf was no larger than that from the 5 per cent mixture. This deterioration of the quality of the protein is ascribed principally to protease activity, and to the presence of the products of partial hydrolysis of the flour proteins.

The flours from the mixtures of wheat germinated 3 days showed progressively large volumes with increasing amounts of germinated wheat. The texture was as good as, or better than the control, up to 20 per cent of germinated wheat. The 40 per cent mixture showed that the quantity of germinated wheat was too large.

Simple nitrogenous compounds including peptids and amino compounds which result from protein hydrolysis were present in larger amounts in the flours milled from the mixtures containing wheat germinated 5 days than from those germinated 3 days. This indicates that proteolysis has proceeded further in the wheat germinated the longer period.

The results of these tests are conclusive evidence that the length of time that wheat germinates, which together with temperature and moisture content determines the length of the epicotyl excerted by the germinating kernel, deserves careful consideration in estimating the effects of germinated wheat upon normal wheat. Not only is the percentage of sprouted kernels important, but also the extent of sprouting. This fact has a bearing upon wheat standardization and the extent to which wheat should be lowered in grade on the basis of the percentage of damaged kernels. The experiments reported here are evidence that 5 per cent of sprouted kernels from a certain lot of wheat may cause deteri-

oration in baking quality, while 20 per cent from another lot may not do so, the difference depending upon the conditions and extent of germination.

In the milling tests on a commercial scale in the Minnesota State Experimental Flour Mill, germinated wheat in amounts as small as 2 and 3 per cent had no appreciable effect upon the milling process. The diastatic activity of the straight grade flour was substantially increased, however, the increase being nearly 100 per cent with the addition of 3 per cent of germinated kernels. It should be possible to aim at a certain level of diastatic power and mill flour very close to that level, providing that the activity of the normal wheat is not too low and the character of the germinated wheat is known. In modern milling practice the protein content of the flour is determined by proper adjustment of the mill mix. These experiments indicate that the diastatic activity of flour can also be approximately adjusted.

Numerous tests were made of the flours thus milled to determine whether significant changes had taken place in characteristics other than diastatic activity. Baking tests both in the laboratory and on a small commercial scale showed definitely that the flours milled from the wheat studied, with admixtures of germinated kernels, were improved in baking value. Larger volumes were obtained, with improved texture, slightly better color of crumb, and better color of crust. The more rapid action of enzymes during fermentation, particularly in the proofing period, shortened the fermentation period.

The changes in volume of a dough and the volume of a gas lost from the dough during fermentation were observed with doughs mixed from each flour. Flours milled from wheat mixtures with 2 and 3 per cent of germinated wheat produced in order more gas and retained more gas than the flour without germinated wheat. This appears to be acceptable evidence that the gas-retaining capacity of the dough was not impaired by the addition of germinated wheat.

In other respects no significant changes were observed. Protease activity studies showed slightly greater activity in the germinated wheat flours upon autolytic digestion for 46 hours, and for 6 days. Fractional nitrogen determinations which indicate hydrolysis of protein gave a slight increase with flours milled from germinated wheat mixtures. The properties of the glutenin as indicated by the viscosity of acidulated flour suspensions were practically the same in all three flours.

The inclusion of 3 per cent or less of germinated wheat caused no measurable change in hydrogen-ion concentration or titratable acidity of the flour.

Studies to determine the effect of aging were made when the flours were a year old and again after 17 and 26 months had elapsed. No appreciable change in diastatic activity was observed at the end of 12 months, but an appreciable decrease was noted at the end of the 26th month. Baking quality improved on aging these flours, the greatest improvement being observed in the flours milled from the wheat mixtures containing germinated kernels.

Conclusions

Diastatic activity of flour has been studied by numerous investigators, and the data in the literature serve to establish the importance of this property of flour in its relation to baking strength. Further evidence of this importance has been afforded by this study.

Germinated wheat may be added to wheat low in diastatic activity to supplement the deficiencies of the latter.

Germination of wheat intended for use in this connection must be conducted under controlled conditions to yield a product of the most serviceable character. Wheat sprouted for 3 days under controlled conditions proved satisfactory; that sprouted for 5 days proved unsuitable, owing, probably, to the hydrolysis of the gluten proteins and to the active proteases which such wheat contributed to the flour.

Catalase activity of the wheat proved to be a useful index of the extent of germination.

Large increases in diastatic activity of flour were effected by the addition of relatively small proportions of germinated wheat. Thus the addition of 2 or 3 per cent of germinated wheat approximately doubled the rate of sugar production in doughs made from the resulting flours. More than 3 per cent did not produce corresponding increases in diastatic activity. It appears probable that from 3 to 5 per cent will give optimum results in mixture with wheats which alone yield flours with a diastatic activity ranging around 100 units. Control of the diastatic activity of flour normally low in that property can be effected within ordinary limits by graduated additions of germinated kernels to the wheat used in the production of the flour.

Milling processes are not interfered with and milling yields are unaffected when 2 or 3 per cent of wheat kernels germinated for 3 days are present in the wheat mixture.

Baking strength of flour milled from wheat low in diastatic activity was increased when 3 per cent of sprouted kernels was added to the wheat. The increased strength was registered in terms of a decidedly increased gas production, a small increase in gas retention, increased loaf volume, and superior grain and texture. Time for proofing or raising the dough in the pan was reduced; color of the crumb appeared whiter, and the crust of the loaves was browner and more pleasing in appearance when the mixture contained germinated wheat.

Protein quality was seemingly unaffected by the addition of 2 or 3 per cent of wheat which had been germinated for 3 days. Protease activity was not increased appreciably, so far as could be detected.

Aging of flour milled from wheat mixtures containing up to 3 per cent of germinated kernels proceeded normally. Baking strength of such flour under ordinary conditions of storage increased somewhat with the lapse of time. Examination of the flour after 26 months of storage showed it to be as sound as, and superior in baking strength to flour milled at the same time from ungerminated wheat.

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Reginald Carter Sherwood was born August 6, 1891, in DeSmet, South Dakota. He attended the public schools there and was graduated from the high school in 1909. In 1910 he entered South Dakota State College, where he specialized in chemistry, receiving the B. S. degree in 1914. As a part time assistant in the department of chemistry in this institution, he pursued advanced study in chemistry, and in 1916 received the M. S. degree. The next three years were spent as assistant chemist in the South Dakota State College with the exception of several month's service during the World War in the Medical Department, U. S. Army. In June 1919, he received the Ph.G. degree from South Dakota State College.

From 1919 to September, 1921, he was analyst for the Montana State Board of Health, and the last year assistant professor in chemistry, Montana State College. His appointment to the staff of the Division of Agricultural Bio-chemistry, University of Minnesota, September, 1921, has continued to the present. He has been enrolled in the Graduate School since 1921. He was appointed Director of the Minnesota State Testing Mill, July 1924.

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